

Digging Behavior of *Solenopsis invicta* Workers When Exposed to Contact Insecticides

JIAN CHEN

USDA-ARS, National Biological Control Laboratory, Biological Control of Pests Research Unit, Stoneville, MS 38776

J. Econ. Entomol. 99(3): 634-640 (2006)

ABSTRACT Contact between ants and insecticides is a prerequisite for contact insecticides to be effective in the control of red imported fire ants, *Solenopsis invicta* Buren. Typically, passive contact occurs in the insecticide application process, but ants also may actively contact insecticides by digging in treated soil or walking on a treated soil surface. Laboratory experiments were conducted to determine whether fire ant workers would dig sand treated with contact insecticides in two different scenarios: 1) no-choice bioassays where insecticide-treated sand was the only available digging substrate, and 2) two-choice bioassays where nontoxicant sand was also available for digging. Eight insecticides that are currently registered in the United States for imported fire ant control were tested. They include acephate, bifenthrin, carbaryl, cyfluthrin, deltamethrin, γ -cyhalothrin, permethrin, and pyrethrin. Workers dug the treated sand for every insecticide tested, even at concentrations up to 10 times of the lowest lethal concentration (LLC) which caused 100% mortality in a toxicity bioassay. However, generally, insecticides significantly reduced the digging effort, even at a concentration that did not cause any significant mortality in the toxicity bioassay.

KEY WORDS digging behavior, red imported fire ant, passive contact, active contact, worker mortality

Contact insecticides are commonly used in controlling the red imported fire ants, *Solenopsis invicta* Buren. Numerous contact insecticides have been tested by broadcast application (Blake et al. 1959, Lofgren et al. 1961, Reinert 1998), individual mound treatment (Appel and Woody 1990, Reinert and Maranz 1996, Jones et al. 1998), and zone treatment (Pranschke et al. 2003). In broadcast application and individual mound treatments using contact insecticide, contact between ant and insecticide is required for the treatments to be effective. There are two possible types of contact between ants and insecticide, passive contact and active contact. Passive contact occurs when insecticide directly reaches ant body during the application process. Active contact happens when ants dig the treated soil and/or walk on the treated soil surface. Active contact may be the only way for some foragers to become exposed to the insecticide. For example, in individual mound treatments, foragers might be outside the mound during the insecticide application, so the only way for them to contact insecticide is to dig the treated mound soil or to walk on the treated surface. Contact insecticide may not be able to reach

some ants inside the mound during application, because of the complexity of the mound structure.

An ideal contact insecticide for fire ant control would be one that does not deter the initial digging behavior even at its lethal concentrations. Such insecticides ensure that ants that miss the passive contact still have an opportunity to achieve active contact. Chen and Allen (2006) successfully demonstrated that workers of red imported fire ant dug the sand treated with lethal doses of fipronil; however, no such information is available on other contact insecticides that are currently registered for fire ant control. The objective of this study was to investigate whether fire ant workers dig sand treated with these contact insecticides. The tested insecticides included eight contact insecticides that are currently registered in the United States for fire ant control: acephate, bifenthrin, carbaryl, cyfluthrin, deltamethrin, γ -cyhalothrin, permethrin, and pyrethrin. Fipronil was excluded because it had been studied in a previous publication (Chen and Allen 2006).

Materials and Methods

Insects. Ant colonies were collected from Pearl River County, Mississippi, on 25 March 2005 and Washington County, Mississippi, on 4 April 2005. Colonies were separated from soil by using a modified method of Banks et al. (1981), with a higher water

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

dropping rate. Ants were reared in a plastic tray (44.5 by 60.0 by 13.0 cm) and Fluon (Ag Fluoropolymers, Chadds Ford, PA) was used to coat the inside wall to prevent ants from escaping. A 500-ml polypropylene square bottle (VWR International, West Chester, PA) with two holes was used as a water supply device. The holes were blocked using cotton balls where ants accessed the water in the bottle. An artificial nest (14-by 2.0-cm petri dish with hardened dental plaster (Castone; Dentsply International Inc., York, PA) on the bottom (1.0 cm in thickness) was placed in each tray. There was a 5.0-cm-diameter brood chamber in the nest. *Helioverpa zea* (Boddie) and tobacco budworm, *Heliothis virescens* (F.), pupae were used as food sources and provided ad libitum in a petri dish. Colonies were maintained in a rearing room at 25–27°C, 58% relative humidity, with a photoperiod of 12:12 (L:D) h.

Insecticides. Acephate (purity, $97.2 \pm 1.0\%$), γ -cyhalothrin (purity, 99.7%), cyfluthrin (mixture of isomers, purity 98.3%), deltamethrin (purity, 99.8%), permethrin (mixture of *cis*- and *trans*-isomers, purity 97.6%), pyrethrin (technical mixture of cinerin I and II, pyrethrin I and II, and jasmolin I and II, purity 21.75%) were obtained from Sigma-Aldrich (St. Louis, MO). Carbaryl (purity, $99 \pm 0.5\%$) and bifenthrin (purity, $99 \pm 0.5\%$) were purchased from Supelco (Bellefonte, PA).

Determination of Worker LC_{50} Values. Mortality of 50 fire ant workers, 24 h after being placed on 40-g insecticide-treated sand in a petri dish (9 cm in diameter, 2 cm in height), was used to calculate the LC_{50} . For each insecticide, six to 11 concentrations were tested, and there were three replicates for each concentration. Fifty worker ants of different sizes were randomly sampled from a colony and placed in each petri dish. The number of dead ants was counted after 24-h exposure to the insecticide. The means of percentage of mortality were subjected to probit analysis to estimate LC_{50} values (SAS Institute 1999). Ants used in this experiment were all from the same colony. Experiments were conducted at room temperature ($22.64 \pm 0.62^\circ\text{C}$). The moisture content of the sand was 8% because Chen (2006) found 4–8% water content in sand was adequate for digging bioassay. Except for acephate, which was dissolved in distilled water, acetone was used as a solvent for all other insecticides. Sand (Premium Play Sand, Plassein International, Longview, TX) was washed using distilled water and dried at 350°C overnight. Treated sand was prepared by placing 120 g of sand in a 1000-ml beaker, adding a 10-ml acetone solution of insecticide into the beaker, and then shaking the beaker to mix the sand with the solution. After acetone was evaporated under a fume hood (40 min), 10.45 ml of distilled water was added into the beaker. Sand in the beaker was stirred with a glass rod to ensure the homogenous mixture of sand and water. For acephate, the water solution was directly added into the sand. The amount of water was adjusted to ensure that the final amount of water was 10.45 ml. The inside wall of the petri dish was coated with Fluon to ensure the contact between ants and the

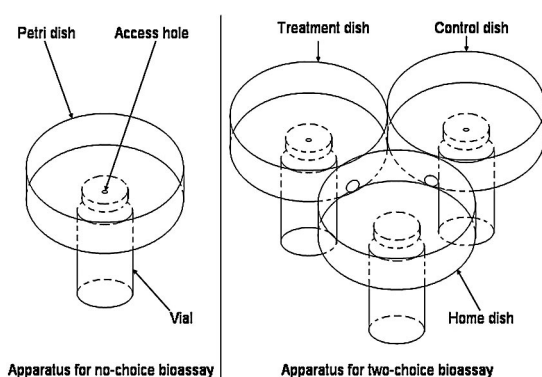


Fig. 1. Diagrams of apparatuses for no-choice and two-choice bioassays.

sand. Concentrations of insecticide evaluated in this study included 0.05, 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.00 ppm for acephate and carbaryl; 0.01, 0.02, 0.04, 0.08, 0.10, 0.2, 0.25, and 0.30 ppm for bifenthrin; 0.001, 0.002, 0.004, 0.006, 0.008, 0.01, 0.012, 0.02, 0.04, 0.06, and 0.08 ppm for cyfluthrin; 0.001, 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.06 ppm for γ -cyhalothrin; 0.01, 0.02, 0.04, 0.06, 0.08, 0.10, and 0.15 ppm for deltamethrin; 0.005, 0.01, 0.02, 0.04, 0.08, 0.10, 0.2, and 0.25 ppm for permethrin; and 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, and 1.00 ppm for pyrethrin. A control treatment contained sand treated only with 10 ml acetone for all of the insecticide tested except acephate which was not treated with any solvent.

No-Choice Digging Bioassays. The bioassay developed by Chen and Allen (2006) was used for the no-choice study. The apparatus consists of one petri dish (9 by 2 cm) and a capped Wheaton liquid scintillation vial (2.8 by 6.1 cm) right underneath it (Fig. 1). There was a 3-mm access hole between the petri dish and the vial. The inside wall of the petri dish was coated with Fluon. The sand preparation method was similar to that described above except 200 g of sand was used. Three concentrations were used: the lowest lethal concentration (LLC) that caused 100% mortality in LC_{50} tests; a concentration that was 10 times the LLC ($LLC \times 10$); and a concentration that was one-tenth of the LLC ($LLC/10$). A mean \pm SD of 36.45 ± 0.75 g sand was added into each vial. Fifty fire ant workers were introduced into each petri dish. After 24 h, sand in each petri dish was collected, dried at 250°C for 12 h, and weighed. Number of ants in the dish and the vial were recorded. There were five replicates for each concentration. Ants used in this experiment were from four different colonies; however, for each insecticide, all ants were from the same colony. The general linear model analysis of variance (ANOVA) and least significant difference (LSD) test (PROC GLM, SAS Institute 1999) were used to compare the amount of sand removed and ant mortality among treatments and controls. Significance was determined at $P < 0.05$. A *t*-test (critical *P* value = 0.05) was used to compare numbers of ants in the dish and vial separately for live and dead ants.

Table 1. LC_{50} values of eight contact insecticides, which were estimated using probit analysis on the means of percentage mortality of 50 fire ant workers, 24 h after being placed on 40-g insecticide-treated sand in a petri dish ($n = 3$)

Insecticide	LC_{50} (ppm)	95% FL	LLC (ppm) ^a	Mortality in control (% \pm SE)
Acephate	0.80	0.76–0.84	1.75	3.33 \pm 2.40
Bifenthrin	32.24×10^{-3}	29.69×10^{-3} – 34.66×10^{-3}	0.08	0.00 \pm 0.00
Carbaryl	1.34	1.31–1.37	2.00	0.67 \pm 0.67
Cyfluthrin	47.19×10^{-3}	45.31×10^{-3} – 48.99×10^{-3}	0.08	1.33 \pm 0.67
γ -Cyhalothrin	10.69×10^{-3}	10.07×10^{-3} – 11.34×10^{-3}	40.00×10^{-3}	0.00 \pm 0.00
Deltamethrin	2.97×10^{-3}	1.39×10^{-3} – 4.81×10^{-3}	60.00×10^{-3}	0.67 \pm 0.67
Permethrin	0.123	0.118–0.130	0.25	0.00 \pm 0.00
Pyrethrin	0.61	0.60–0.62	1.00	0.00 \pm 0.00

^a Lowest lethal concentration that caused 100% mortality in all replicates (ppm).

Two-Choice Digging Bioassays. The bioassay method of Chen and Allen (2006) was used for the two-choice study. The apparatus consisted of three laterally connected petri dishes (9-cm \times 2-cm): a home dish, treatment dish, and control dish (Fig. 1). These petri dishes were connected together using glue (Arrow Fastener Co., inc., Saddle Brook, NJ) and a capped Wheaton liquid scintillation vial (2.8 cm \times 6.1 cm) was placed under each petri dish. At the center of the treatment and control dishes, a 3-mm access hole was drilled through the vial cap underneath. There were two holes (0.5 cm diameter), each on the connection point between home dish and other two dishes. The inside wall of all petri dish was coated with Fluon. The sand preparation method was the same as described above. Insecticide-treated sand was placed in the vial under the treatment dish and untreated sand in vial under the control dish. Fifty fire ant workers were introduced into the home dish. The $LLC \times 10$ was the only concentration evaluated in two-choice bioassay. The experiment was conducted at 23°C. After 24 h, sand in each petri dish was collected, dried at 250°C for 12 h, and weighed. Sand in each vial was collected if there was sand in home dish. The amount of sand removed was calculated from the weight difference before and after the digging experiment. The number of ants in each dish and vial was recorded. A paired *t*-test (critical *P* value = 0.05) was used to compare mean amount of sand removed and numbers of ants in the treatment vial with those in the control vial. Ants used in this experiment were from the same colony.

Results

Worker LC_{50} Values. The LC_{50} values of eight contact insecticides on fire ant workers are presented in Table 1. In no cases did control mortality exceed 5.0%. Among the eight insecticides, carbaryl was the least toxic to fire ant workers and deltamethrin was the most toxic. LC_{50} values were all different because no overlap of fiducial limits existed among LC_{50} values.

No-Choice Digging Bioassays. The amount of sand removed and worker mortality are summarized in Table 2. Workers dug treated sand in every case, even at the $LLC \times 10$. However, digging behavior was significantly reduced even at $LLC/10$, which was lower

than the highest concentrations that did not cause any significant mortality in LC_{50} tests. Mortality at the LLC was always significantly lower than that at $LLC \times 10$. Mortality at LLC was significantly higher than that at $LLC/10$ for acephate, bifenthrin, carbaryl, cyfluthrin, γ -cyhalothrin, and deltamethrin; however, there was no significant difference in mortality between LLC and $LLC/10$ for permethrin and pyrethrin. Difference in mortality between $LLC/10$ and control was not significant for all insecticides. Significantly more sand was removed at control than that at LLC for all insecticides. The amount of removed sand at the LLC was significantly higher than that at the $LLC \times 10$ for cyfluthrin, γ -cyhalothrin, deltamethrin, and pyrethrin. However, for the rest of insecticides, there was no difference in amount of removed sand between these two concentrations. Only acephate, carbaryl, and deltamethrin achieved 100% mortality at the $LLC \times 10$. The rest of the insecticides caused only 12 to 90% mortality at the $LLC \times 10$ level. In controls, after 24 h the majority of live ants were found in the vial and dead ants, if any, were all in the dish (Table 3). At the LLC dead ants were found in the vial for all insecticides except acephate and pyrethrin. At $LLC \times 10$, the majority of live ants, if any, were in the dish. The majority of dead ants were also in the dish, although dead ants were found in vials for all insecticides except acephate.

Two-Choice Digging Bioassays. The amount of removed sand and worker mortality are summarized in Table 4. For all eight insecticides, workers dug into the treated sand; however, except for acephate, workers removed significantly more sand from the control vial than that from the treated vial. No insecticide caused 100% mortality. Acephate and bifenthrin were two insecticides that achieved the highest mortality (80.60 and 46.00%, respectively), whereas pyrethrin had the lowest mortality (4.33%). For each insecticide, significantly more dead ants were in the treatment (dish + vial), whereas significantly more surviving ants were in the control (dish + vial) (Table 5).

Discussion

For all eight insecticides, workers dug the treated sand in both no-choice and two-choice bioassays. This indicates that fire ant workers may have opportunity

Table 2. Worker mortality and amt of removed sand 24 h after 50 workers were released in no-choice digging bioassay apparatus ($n = 5$)

Insecticide	Concn (ppm)	Sand removed (g, mean \pm SE)	Mortality (% , mean \pm SE)
Acephate	Control	1.14 \pm 0.13a	1.60 \pm 0.75c
	0.175 (LLC/10)	0.65 \pm 0.17b	2.00 \pm 0.89c
	1.75 (LLC)	0.15 \pm 0.04c	26.00 \pm 5.06b
	17.50 (LLC \times 10)	0.05 \pm 0.01c	100.00 \pm 0.00a
Bifenthrin	Control	2.62 \pm 0.31a	0.80 \pm 0.49c
	0.008 (LLC/10)	1.37 \pm 0.14b	0.80 \pm 0.49c
	0.08 (LLC)	0.37 \pm 0.02c	8.80 \pm 1.74b
	0.8 (LLC \times 10)	0.05 \pm 0.01c	90.00 \pm 2.10a
Carbaryl	Control	2.06 \pm 0.17a	4.00 \pm 1.41c
	0.20 (LLC/10)	1.49 \pm 0.19b	10.40 \pm 3.12c
	2.00 (LLC)	0.27 \pm 0.02c	28.40 \pm 3.54b
	20.0 (LLC \times 10)	0.03 \pm 0.01c	100.00 \pm 0.00a
Cyfluthrin	Control	2.02 \pm 0.099a	2.40 \pm 0.49c
	0.008 (LLC/10)	0.80 \pm 0.107b	2.80 \pm 0.74c
	0.08 (LLC)	0.39 \pm 0.024c	13.20 \pm 4.13b
	0.80 (LLC \times 10)	0.034 \pm 0.005d	52.80 \pm 3.20a
γ -Cyhalothrin	Control	2.43 \pm 0.05a	3.20 \pm 1.86c
	0.004 (LLC/10)	0.63 \pm 0.09b	3.40 \pm 1.40c
	0.04 (LLC)	0.26 \pm 0.04c	18.00 \pm 1.67b
	0.40 (LLC \times 10)	0.03 \pm 0.01d	86.00 \pm 3.10a
Deltamethrin	Control	2.43 \pm 0.05a	3.20 \pm 1.86c
	0.006 (LLC/10)	0.58 \pm 0.05b	1.60 \pm 0.75c
	0.06 (LLC)	0.21 \pm 0.02c	23.00 \pm 3.12b
	0.60 (LLC \times 10)	0.06 \pm 0.02d	100.00 \pm 0.00a
Permethrin	Control	1.79 \pm 0.12a	6.40 \pm 2.32b
	0.025 (LLC/10)	1.17 \pm 0.05b	6.80 \pm 1.63b
	0.25 (LLC)	0.25 \pm 0.07c	11.60 \pm 1.60b
	2.50 (LLC \times 10)	0.07 \pm 0.03c	42.00 \pm 6.07a
Pyrethrin	Control	2.82 \pm 0.17a	0.40 \pm 0.40b
	0.10 (LLC/10)	0.73 \pm 0.07b	0.40 \pm 0.40b
	1.00 (LLC)	0.55 \pm 0.05b	2.00 \pm 0.89b
	10.00 (LLC \times 10)	0.05 \pm 0.02c	12.00 \pm 2.61a

LLC, lowest lethal concentration (ppm) that caused 100% mortality in all replicates in the LC₅₀ test.

The general linear model analysis of variance and LSD test were used to compare the amount of sand removed and ant mortality among treatments and controls. Means with different letters were significantly different ($P < 0.05$).

to actively contact soil treated with these insecticides, even at concentrations that were lethal to fire ants. Pyrethroid insecticides were considered as “repellent and fast-acting contact insecticides” when used in surface treatments in and around structures, in contrast to the fipronil that was labeled as “nonrepellent slow-acting contact insecticide.” Digging behavior of fire ant workers on sand treated with pyrethroids, such as bifenthrin, cyfluthrin, deltamethrin, γ -cyhalothrin, and permethrin, suggested that these insecticides might not repel ants but merely prevent ants from digging further by killing or weakening some ants, which might signal other ants to stop digging. How many ants can achieve active contact may depend on how rapidly the insecticide acts, which is most likely related to insecticide concentration in the soil.

To relate the concentrations of contact insecticides used in this study to their application rates recommended in product labels, one insecticide product for each active ingredient was chosen to calculate the insecticide concentration in the soil. Acephate 75 WSP (United Phosphorus Inc., Trenton, NJ) was selected for acephate; Talstar GC Granular Insecticide (FMC Corporation, Agricultural Products Group, Philadelphia, PA) for bifenthrin; carbaryl 10% Dust (Southern Agricultural Insecticides, Inc., Boone, NC) for carbaryl; Fire Ant Control Granules (Southern

Agricultural Insecticides, Inc.) for permethrin; Bayer Advanced Lawn Fire Ant Killer (Bayer Advanced LLC, Brimingham, AL) for cyfluthrin; Bengal Ultra Dust 2X Fire Ant Killer (Bengal Products, Inc., Baton Rouge, LA) for deltamethrin; Spectracide Fire ant Mound & Broadcast Granule₂ (Chemsico, Division of United Industries Corporation, St. Louis, MO) for γ -cyhalothrin; and Organic One Fire Ant Insecticides (Organic One, Altha, FL) for pyrethrin. The recommended application rates plus the following four assumptions were used to calculate insecticide concentration in the soil: 1) fire ant mound is a hemisphere; 2) the average diameter of the hemisphere is 0.381 m (Sherman and Cokendolpher 1988); 3) after being applied on the surface of the soil, insecticide will penetrate 1.0 cm into the soil; and 4) weight of 1.0 cm of top soil is 1.0 g/cm³. The following values are the estimated concentration for each contact insecticide in 1.0 cm of top soil: acephate, 341.20 ppm; bifenthrin, 2.25–4.49 ppm; carbaryl, 97.65 ppm; permethrin, 4.88–7.32 ppm; cyfluthrin, 2.84 ppm; deltamethrin, 0.36 ppm; γ -cyhalothrin, 3.65–3.95 ppm; and pyrethrin, 1.36–1.67 ppm.

Except pyrethrin in Organic One Fire Ant Insecticide, the estimated concentration of each contact insecticide in the soil was much higher than that used in this study. To exploit the active contact, the applica-

Table 3. Number of live and dead workers in dish and vial of no-choice digging bioassay apparatus 24 h after workers were released in the dish (mean \pm SE, $n = 5$)

Insecticide	Concn	Dish		Vial	
		Live	Dead	Live	Dead
Acephate	Control	3.60 \pm 1.60	0.80 \pm 0.37	45.60 \pm 1.54*	0.00 \pm 0.00
	0.175	7.60 \pm 1.86	1.00 \pm 0.45	40.40 \pm 2.25*	1.00 \pm 0.32
	1.75	6.00 \pm 1.14	13.00 \pm 2.53	31.00 \pm 2.35*	0.00 \pm 0.00*
	17.5	0.00 \pm 0.00	50.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00*
Bifenthrin	Control	0.00 \pm 0.00	0.40 \pm 0.25	49.60 \pm 0.25*	0.00 \pm 0.00
	0.008	2.80 \pm 0.80	0.40 \pm 0.25	47.20 \pm 0.80*	0.00 \pm 0.00
	0.08	34.20 \pm 1.83	4.20 \pm 0.86	11.40 \pm 1.08*	0.20 \pm 0.20*
	0.80	4.20 \pm 0.49	37.00 \pm 2.32	0.00 \pm 0.00*	9.00 \pm 2.28*
Carbaryl	Control	0.00 \pm 0.00	0.00 \pm 0.00	50.00 \pm 0.00*	0.00 \pm 0.00
	0.20	0.00 \pm 0.00	0.20 \pm 0.20	49.80 \pm 0.20*	0.00 \pm 0.00
	2.00	0.00 \pm 0.00	18.20 \pm 1.53	30.60 \pm 1.50*	1.20 \pm 0.37*
	20.00	0.00 \pm 0.00	46.80 \pm 0.67	0.00 \pm 0.00	3.20 \pm 0.67*
Cyfluthrin	Control	0.80 \pm 0.58	1.40 \pm 0.25	47.80 \pm 0.74*	0.00 \pm 0.00*
	0.008	4.60 \pm 1.81	1.20 \pm 0.37	44.40 \pm 1.75*	0.00 \pm 0.00*
	0.08	17.00 \pm 2.10	6.60 \pm 2.07	23.40 \pm 1.99	3.00 \pm 1.27
	0.80	11.40 \pm 1.33	26.40 \pm 2.60	1.40 \pm 0.87*	8.80 \pm 2.06*
γ -Cyhalothrin	Control	1.20 \pm 0.37	1.60 \pm 0.93	47.20 \pm 0.67*	0.00 \pm 0.00
	0.004	0.60 \pm 0.40	1.80 \pm 0.87	47.60 \pm 0.81*	0.00 \pm 0.00*
	0.04	20.60 \pm 1.81	8.20 \pm 0.87	20.40 \pm 1.91	0.80 \pm 0.37*
	0.4	7.00 \pm 1.55	38.60 \pm 1.03	0.00 \pm 0.00*	4.40 \pm 1.03*
Deltamethrin	Control	1.20 \pm 0.37	1.60 \pm 0.93	47.20 \pm 0.66*	0.00 \pm 0.00
	0.006	0.20 \pm 0.20	0.80 \pm 0.37	49.00 \pm 0.32*	0.00 \pm 0.00
	0.06	16.40 \pm 3.22	11.60 \pm 1.63	23.80 \pm 3.32	0.20 \pm 0.20*
	0.6	3.80 \pm 1.39	43.80 \pm 1.96	0.00 \pm 0.00*	4.40 \pm 0.68*
Permethrin	Control	9.60 \pm 1.69	3.20 \pm 1.16	37.20 \pm 2.33*	0.00 \pm 0.00*
	0.025	5.80 \pm 1.39	3.40 \pm 0.81	40.80 \pm 1.16*	0.00 \pm 0.00*
	0.25	13.00 \pm 1.67	3.80 \pm 0.49	31.20 \pm 2.27*	2.00 \pm 0.45*
	2.50	28.00 \pm 2.97	15.00 \pm 2.35	1.40 \pm 0.60*	5.60 \pm 1.40*
Pyrethrin	Control	0.00 \pm 0.00	0.20 \pm 0.20	49.80 \pm 0.20*	0.00 \pm 0.00
	0.10	0.00 \pm 0.00	0.00 \pm 0.00	50.00 \pm 0.00*	0.00 \pm 0.00
	1.00	13.60 \pm 2.58	1.00 \pm 0.45	35.40 \pm 2.52*	0.00 \pm 0.00
	10.00	44.00 \pm 1.30	5.40 \pm 1.33	0.00 \pm 0.00*	0.60 \pm 0.60*

* Number of ants in the vial was significantly different from that in the dish at the same concn (t -test, $P < 0.05$).

tion rates for these products may have to be significantly reduced. However, in addition to the active ingredient, other components in a formulation may affect digging behavior. The effect of a commercial product on fire ant digging behavior can only be evaluated using commercial formulation rather than just pure active ingredient.

Because workers in no-choice bioassays were not from the same colony, any comparisons among insecticides may not be adequate. However, because workers were randomly sampled from the same colony for a particular insecticide, any comparison in one bioassay is valid. Significant difference in the amount of removed sand among treatments and controls in no-

choice bioassays might be explained by the lethal and sublethal toxicity of the insecticides. At the sublethal concentrations, such as the LLC/10 concentrations for all eight tested insecticides, no significant mortality was observed. Nevertheless, ants might have been significantly weakened, resulting in a significantly lower amount of sand being removed from the arena. However, differences between treatment and control in two-choice bioassays could not be explained solely by the toxicity. When ants had equal chance to dig sand in treatment and control arenas, they would dig equal amount of sand from both arenas, even when they had been intoxicated by the insecticide. Such differences might be explained by labor allocation. In

Table 4. Worker mortality and amount of removed sand 24 h after 50 workers were released in the home dish of two-choice bioassay apparatus ($n = 10$)

Insecticide	Concn (ppm)	Sand removed (g, mean \pm SE)			% mortality (mean \pm SE)
		Treated sand	Untreated sand	P value of t -test	
Acephate	17.50	0.31 \pm 0.09	0.48 \pm 0.13	0.42	80.60 \pm 6.93
Bifenthrin	0.80	0.03 \pm 0.01	0.24 \pm 0.01	<0.0001	46.00 \pm 3.80
Carbaryl	20.00	0.08 \pm 0.01	0.57 \pm 0.19	0.039	31.11 \pm 4.98
Cyfluthrin	0.80	0.06 \pm 0.01	0.58 \pm 0.05	<0.0001	22.2 \pm 2.61
γ -Cyhalothrin	0.40	0.14 \pm 0.02	0.21 \pm 0.02	0.0016	15.50 \pm 2.98
Deltamethrin	0.60	0.06 \pm 0.01	0.30 \pm 0.01	0.0014	30.73 \pm 4.90
Permethrin	2.50	0.05 \pm 0.01	0.40 \pm 0.04	<0.0001	16.00 \pm 2.86
Pyrethrin	10.00	0.01 \pm 0.003	0.76 \pm 0.10	<0.0001	4.33 \pm 1.63

Table 5. Number of live and dead workers in control and treatment of two-choice digging bioassay 24 h after workers were released in the home dish (mean \pm SE, $n = 10$)

Insecticide	Live/dead	Control (dish + vial)	Treatment (dish + vial)	P value (paired <i>t</i> -test)
Acephate	Live	9.70 \pm 3.46	0.00 \pm 0.00	0.02
	Dead	11.60 \pm 1.69	22.80 \pm 2.94	0.0068
Bifenthrin	Live	24.10 \pm 2.94	1.30 \pm 0.65	<0.0001
	Dead	3.10 \pm 1.16	15.40 \pm 1.78	0.0003
Carbaryl	Live	33.22 \pm 0.35	0.89 \pm 0.39	<0.0001
	Dead	0.78 \pm 0.36	13.22 \pm 2.41	0.0015
Cyfluthrin	Live	37.80 \pm 1.30	1.10 \pm 0.41	<0.0001
	Dead	0.20 \pm 0.13	10.10 \pm 1.39	<0.0001
γ -Cyhalothrin	Live	38.10 \pm 2.19	2.60 \pm 1.20	<0.0001
	Dead	0.50 \pm 0.22	7.90 \pm 1.20	<0.0001
Deltamethrin	Live	31.60 \pm 2.50	0.80 \pm 0.33	<0.0001
	Dead	1.60 \pm 0.37	12.20 \pm 1.83	0.0001
Permethrin	Live	40.40 \pm 1.46	0.30 \pm 0.15	<0.0001
	Dead	0.50 \pm 0.34	7.20 \pm 1.41	0.001
Pyrethrin	Live	47.30 \pm 0.91	0.10 \pm 0.10	<0.0001
	Dead	0.10 \pm 0.10	2.10 \pm 0.80	0.034

two-choice bioassays, the digging task might be allocated at two locations: treatment and control. Ants might be grouped somehow based on digging sites. An ant that initially dug in the control most likely would dig in the control for the remainder of the bioassay period. Consequently, ants grouped on treatment location were more likely to be intoxicated or killed, which would lead to significantly less treated sand being removed from the vial. Labor allocation also was supported by the ant distribution data in the bioassay apparatus: more dead ants were found in the treatment and more live ants in the control. The same rationale might explain the fact that not a single insecticide at $LLC \times 10$ in two-choice bioassays achieved 100% worker mortality. Some ants that initially dug into the sand in a control vial might never be exposed to insecticides. Transient division of labor, the short-term adherence to a specific task to the exclusion of other tasks, was demonstrated in other ant species, such as cooperative prey retrieval in *Formica schaufussi* (Robson and Traniello 2002). However, whether the transient digging tasks in *S. invicta* are indeed allocated by digging sites can only be answered by further experiments.

Worker ants removed from a colony may behave differently from ants in an intact colony. General fitness also may vary from one ant colony to another. To more closely simulate the effect of insecticide on digging behavior in the field, intact colonies from different locations must be used for future studies.

Acephate is the only insecticide that did not cause significant differences in the amount of sand removed between treatment and control in the two-choice bioassay. Acephate is the only water-soluble organophosphate among the eight insecticides tested in this study. This may justify further investigation of water-soluble organophosphates for fire ant control.

Chemical control using contact insecticides will continue to be a critical component in fire ant management. Unlike fire ant bait technology, which has attracted more attention from entomologists attempting to maximize the bait efficacy by exploiting fire ant behavior, few researchers have focused on enhancing

the efficacy of contact insecticides by exploiting ant behavior. Incorporating chemicals that elicit ant digging behavior into contact insecticide formulations may prove to be a promising line of research.

Acknowledgments

I thank Drs. Douglas A. Streett and Eric Riddick (USDA-ARS, Stoneville, MS) for providing valuable comments on this manuscript, and Gordon Andrews and Jim Robbins (Delta Research and Extension Center, Stoneville, MS) for critical reviews of the manuscript. I thank Xikui Wei for assistance in preparing the bioassays.

References Cited

- Appel, A. G., and L. G. Woody. 1990. Individual mound treatment for rapid control of fire ants, pp. 248–251. In L. E. Bode, J. L. Hazen, and D. G. Chasin [eds.], Pesticide formulations and application systems, ASTM STP 1078, vol. 10. Philadelphia, PA.
- Banks, W. A., C. S. Lofgren, D. P. Jouvenaz, C. E. Stringer, P. M. Bishop, D. F. Williams, D. P. Wojcik, and B. M. Glancey. 1981. Techniques for collecting, rearing, and handling imported fire ants. U.S. Dep. Agric. SEA Adv. Agric. Tech. AAT-S-21/April 1981.
- Blake, G. H., Jr., W. G. Eden, and K. L. Hays. 1959. Residual effectiveness of chlorinated hydrocarbons for control of the imported fire ant. J. Econ. Entomol. 52: 1–3.
- Chen, J. 2005. Assessment of repellency of nine phthalates against red imported fire ant (Hymenoptera: Formicidae) workers using ant digging behavior. J. Entomol. Sci. 40: 368–377.
- Chen, J., and M. L. Allen. 2006. Significance of digging behavior to mortality of red imported fire ant workers, *Solenopsis invicta* Buren, in fipronil treated sand. J. Econ. Entomol. 99: 476–482.
- Jones, D. B., L. C. Thompson, and K. W. Davis. 1998. Use of fenoxycarb followed by acephate for spot eradication of imported fire ants (Hymenoptera: Formicidae). J. Kans. Entomol. Soc. 70: 169–174.
- Lofgren, C. S., V. E. Adler, and W. F. Barthel. 1961. Effect of some variations in formulation or application procedure on control of the imported fire ant with granular heptachlor. J. Econ. Entomol. 54: 45–47.

- Pranschke, A. M., L. M. Hooper-Bui, and B. Moser. 2003. Efficacy of bifenthrin treatment zones against red imported fire ant. *J. Econ. Entomol.* 96: 98–105.
- Reinert, J. A. 1998. Broadcast treatment of granular insecticides for control of the red imported fire ant in urban landscapes, 1996. *Arthropod Manage. Tests* 23: 325–326.
- Reinert, J. A. and S. J. Maranz. 1996. Evaluation of Delta-methrin granules for individual mound control of red imported fire ant in residential landscapes, 1995. *Arthropod Manage. Tests* 21: 368.
- SAS Institute. 1999. SAS/STAT user's guide version 8. SAS Institute, Cary, NC.
- Sherman, A. P., Jr., and J. C. Cokendolpher. 1988. Environmental limitation on the imported fire ant and the ants' response to environmental changes, pp. 11–17. *In* S. B. Vinson and J. Teer [eds.], *The imported fire ant: assessment and recommendations*. Proceedings of Governor's Conference, Sportsmen Conservations of Texas, Austin, TX.
- Robson, S.K.A., and J.F.A. Traniello. 2002. Transient division of labor and behavioral specialization in the ant *Formica schaufussi*. *Naturwissenschaften* 89: 128–131.

Received 27 September 2005; accepted 14 February 2006.
